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# Refining the Primrose Syndrome Phenotype: a Study of Five Patients with ZBTB20 De Novo Variants and a Review of the Literature

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## ABSTRACT

**Primrose syndrome is a rare autosomal dominant condition caused by heterozygous missense variants within *ZBTB20.* Through an exome sequencing approach (as part of the Deciphering Developmental Disorders (DDD) study) we have identified five unrelated individuals with previously unreported, *de novo ZBTB20* pathogenic missense variants. All five missense variants targeted the C2H2 zinc finger domains. This genotype-up approach has allowed further refinement of the Primrose syndrome phenotype. Major characteristics (>90% individuals) include an intellectual disability (most frequently in the moderate range), a recognizable facial appearance and brain MRI abnormalities, particularly abnormalities of the corpus callosum. Other frequent clinical associations (in 50-90% individuals) include sensorineural hearing loss (83%), hypotonia (78%), cryptorchidism in males (75%), macrocephaly (72%), behavioral issues (56%) and dysplastic/hypoplastic nails (57%). Based upon these clinical data we discuss our current management of patients with Primrose syndrome.**

**Key words: Primrose syndrome, ZBTB20, exome sequencing, DDD study, intellectual disability**

## INTRODUCTION

Primrose syndrome (OMIM 259050), first described in 1982 by David Primrose, has previously been associated with a moderate to severe intellectual disability, a recognizable

facial appearance with deep set eyes, narrow, often down-slanting palpebral fissures, ptosis, depressed nasal bridge and macrocephaly with or without tall stature [Primrose, 2008, Collacott et al., 2008, Lindor et al., 1996, Battisti et al., 2002, Mathijssen et al., 2006, Dalal et al., 2010, Posmyk et al., 2011, Carvalho et al., 2011, Mattioli et al., 2016, Casertano et al., 2017, Stellacci et al., 2018, Cordeddu et al., 2014, Alby et al., 2018]. Additional reported clinical features include muscle wasting, calcified pinnae, hearing loss, cataracts, hypothyroidism [Dalal et al., 2010, Mattioli et al., 2016], torus palatinus (a benign osseous elevation usually found on the midline of the hard palate) [Primrose, 2008, Collacott et al., 2008, Mathijssen et al., 2006, Dalal et al., 2010], and sparse body and facial hair [Primrose, 2008, Collacott et al., 2008, Lindor et al., 1996, Battisti et al., 2002, Mathijssen et al., 2006, Carvalho et al., 2011].

In 2014, heterozygous pathogenic variants within *ZBTB20* were shown to cause Primrose syndrome [Cordeddu et al., 2014]. *ZBTB20,* located at chromosome position 3q13.31, encodes one of a family of POK (POZ (pox virus and zinc finger) and Kruppel) proteins which acts as a transcriptional repressor and has a role in glucose metabolism, postnatal growth and neurogenesis [Sutherland et al., 2009, Zhang et al., 2012, Xie et al., 2010]. ZBTB20 has five C2H2 zinc finger domains (ZNFI-ZNFV) and an N-terminal BTB (Broad Complex, Tramtrack, Bric a brac) domain that mediate interaction with DNA (figure 1a)[Sutherland et al., 2009].

To date, 14 of 15 Primrose syndrome reported *ZBTB20* pathogenic variants are missense variants clustering within the first (three variants), second (six variants) and third (two variants) zinc finger domains and the linker region between the first two motifs (three variants, figure 1a, supplementary table 1)[Mattioli et al., 2016, Casertano et al., 2017,

Stellacci et al., 2018, Cordeddu et al., 2014, Alby et al., 2018]. Functional assays have supported a dominant negative mechanism of disease whereby missense variants result in a stable but dysfunctional protein with defective DNA binding [Cordeddu et al., 2014]. In contrast, haploinsufficiency of *ZBTB20* causing the 3q13.31 microdeletion syndrome (OMIM 615433), has been reported to cause a similar but distinct condition also characterized by increased growth but without many of the Primrose syndrome clinical associations including the recognizable facial appearance, calcified pinnae and muscle wasting [Molin et al., 2012, Shuvarikov at al., 2013].

Here we report five patients with *de novo ZBTB20* pathogenic missense variants, identified through trio-based exome sequencing. This genotype-up approach has replicated the previous finding that *ZBTB20* missense variants target the zinc finger domains and has allowed a non-biased refinement of the Primrose syndrome phenotype.

## METHODS

The study was approved by the UK Research Ethics Committee (10/H0305/83), granted by the Cambridge South Research Ethics Committee. Informed consent was obtained from all families. Seven patients with *de novo ZBTB20* variants were identified through the Deciphering Developmental Disorders (DDD) Study using a trio-based exome sequencing strategy and methods as previously described [Firth et al., 2009]. Five patients had missense variants, predicted to be pathogenic according to American College of Medical Genetics and Genomics (ACMG criteria PS2, PM1, PM2, PP2 and PP3) [Richards et al., 2015] with evidence detailed in supplementary table 2. These five missense variants targeted the first, second and third zinc fingers (ZNFI, ZNFII and ZNFIII). None had previously been reported.

The remaining two patients with *de novo ZBTB20* variants were not included in the current study: one patient with a c.505G>C; p.(Glu169Gln) variant because there was insufficient evidence to support pathogenicity of the variant and the other patient with a with a c.1020C>G; p.(Tyr340*) variant because, in addition to lack of evidence for variant pathogenicity in Primrose syndrome (PVS1 will not apply), the patient had a maternally inherited *FLNA* likely pathogenic variant resulting in a compound phenotype. It was therefore unclear which clinical features were attributable to the *ZBTB20* variant and which to the *FLNA* variant.

Clinical data for the five patients with the single *de novo* pathogenic missense *ZBTB20* variants were obtained through face to face review by one of the authors, all experienced dysmorphologists, and a standardized proforma. Growth parameter z scores were calculated with reference to the CDC (Centers for Disease Control and Prevention) data other than BMI z scores where we calculated z scores with reference to WHO (World Health Organization) data. Photographs, with accompanying consent to publish, were received from all five families.

## RESULTS

Clinical details are summarized in table 1. Detailed case reports are shown below:

Patient 1 (DDD 273936)

Patient 1, female, had a *de novo ZBTB20* c.1749C>G p.(Cys583Trp) pathogenic variant. She was born at 38 weeks gestation following an uncomplicated pregnancy. Her birth weight was 2.9kg (-1.0 standard deviations below the mean, -1.0SD) with a head circumference of 36cm (+0.8SD). At the age of 3 years, her height was 99cm (+1.2SD),

weight was 15kg (-0.6SD), BMI was 15.3kg/m$^2$ (-0.1SD) and head circumference was 51.5cm (+1.9SD). Patient 1 had a severe learning disability and was delayed in the attainment of her developmental milestones: she sat between 6 and 7 years of age, crawled aged 7 years, she was not walking when reviewed at the age of 9 years and remained non-verbal. She had hypothyroidism, hypotonia and brachycephaly. A brain MRI scan identified colpocephaly and agenesis of the corpus callosum (figure 1c).

Patient 2 (DDD 303448)

Patient 2, male, had a *de novo ZBTB20* c.1850T>C p.(Leu617Ser) pathogenic variant. He was one of dizygotic twins born at 37+6 weeks gestation following an uncomplicated pregnancy. Birth weight was 2.3kg (-2.0SD) with a head circumference of 35.4cm (-0.2SD). At 3.9 years, his height was 97cm (-1.1SD), weight was 12.3kg (-2.5SD), BMI was 13.1 kg/m$^2$ (-2.0SD) and head circumference was 53.5cm (+2.3SD). Patient 2 sat unsupported at 21 months, was not able to walk (at 3.9 years) and began to babble at 34 months. He would head bang when frustrated. Patient 2 had a moderate-severe congenital sensorineural hearing loss, a small patent foramen ovale and generalised hypotonia, more prominent in the lower limbs. He had had one focal seizure. He had hypermetropia, astigmatism and a convergent squint. Dysmorphic features included low-set, posteriorly rotated ears, flat mid-face with prominent forehead, deep-set eyes with down slanting and narrow palpebral fissures and a thin upper lip. A brain MRI scan demonstrated partial agenesis of the corpus callosum (figure 1c).

Patient 3 (DDD 273033)

Patient 3, female, had a *de novo ZBTB20* c.1879A>G p.(Thr627Ala) pathogenic variant. She was born at 39 weeks gestation following a normal pregnancy with a birth

weight of 2.98kg (-0.8SD). There were some difficulties establishing feeding in the early neonatal period but no other significant concerns. At the age of 2.3 years, her height was 85cm (-0.8SD), weight was 10.9kg (-1.4SD), BMI was 15.1 kg/m$^2$ (-0.4SD) and head circumference was 50.5 cm (+1.9SD). Patient 3 was slow to reach her developmental milestones; she sat at 6 months, walked aged 3-4 years, and spoke her first words between 2.5 and 3 years. She had a moderate learning disability. She had mild generalized hypotonia in infancy progressing to truncal hypotonia in childhood. Additional medical problems included thoracolumbar scoliosis, mixed conductive and sensorineural hearing loss, hypermetropia with recurrent blepharoconjunctivitis and raised urinary calcium with possible nephrocalcinosis. Patient 3 had joint hypermobility with soft, doughy skin, cutis marmorata and dysplastic nails. She had a prominent forehead, narrow mouth with thin lips, pointed chin, and upslanting, narrow palpebral fissures (figure 1b). Dentition was poor with delayed secondary dentition. A brain MRI scan was unremarkable and bone age was delayed (the bone age was 2 years at chronological age of 3 years 4 months).

Patient 4 (DDD 263871)

Patient 4, male, had a *de novo* mosaic *ZBTB20* c.1943C>T p.(Ser648Phe) pathogenic variant with a variant allele fraction of 0.377 on DNA derived from saliva (50X read depth) and 0.332 on DNA derived from blood (100,000X read depth, DDD personal communication). He was born at term following an uncomplicated pregnancy with a birth weight of 5.3kg (+3.9SD). At 11.25 years, his height was 154.5cm (+1.3SD), weight was 73.7kg (+2.6SD), BMI was 30.9kg/m$^2$ (+3.4SD) and head circumference was 61cm (+5.3SD). He sat at 7 months, walked at 12 months and developed speech at 3-4 years.

He had a moderate learning difficulty. He was a poor sleeper, had a poor working memory and a tendency to temper tantrums. He had bilateral cryptorchidism, a convergent squint and sensorineural hearing loss. He had narrrow, downslanting palpebral fissures and a high arched palate (figure 1b). A brain MRI scan identified a Chiari malformation.

Patient 5 (DDD 280375)

Patient 5, male, had a *de novo ZBTB20* c.1967A>G p.(His656Arg) pathogenic variant. He was born at 41+5 weeks gestation following an uncomplicated pregnancy. He was delivered via an emergency caesarean section for fetal distress and had a birth weight of 2.6kg (-1.6SD). There were no immediate postnatal concerns. At 13.4 years of age, his height was 154cm (-0.6SD), weight was 68.3kg (+1.6SD), BMI was 28.8kg/$m^2$ (+2.5SD) and head circumference was 59cm (+3.1SD). He sat at 10 months, walked aged 3-4 years, and developed his first words between 2.5 and 3 years of age. He had a moderate learning difficulty. He had moderate bilateral congenital sensorineural hearing loss, obesity (associated with hyperphagia), central and peripheral hypotonia and a marked lumbar lordosis. He had mild camptodactyly, hypoplastic 5th toe nails, and acrocephaly. Dysmorphic features included narrow, downslanting palpebral fissures, prominent ears and a thin upper lip (figure 1b). A brain MRI scan demonstrated a thin corpus callosum.

## DISCUSSION

Through an exome sequencing approach, the current study has identified five patients with novel single *de novo ZBTB20* missense variants. These data replicate previous reports that Primrose syndrome missense variants cluster within the zinc finger

domains and expand the clustering to include the third zinc finger domain where only two pathogenic variants had previously been reported [Stellacci et al., 2018, Alby et al., 2018]. Stellachi et al., recently reported a frameshift variant outside of the zinc finger domains said to cause Primrose syndrome. Given the findings both from the current study and previously reported studies, a frameshift variant would be an unusual cause of Primrose syndrome. In addition, further evaluation of the phenotype in this patient suggests greater similarity with the 3q13.31 microdeletion syndrome than Primrose syndrome with increased growth and a milder intellectual disability than that normally described in Primrose syndrome. In addition, this patient, facially, bears a significant resemblance to the patient reported by Rasmussen et al., with a 3q13.31 microdeletion, rather than patients with Primrose syndrome.

The identification of five additional patients with pathogenic *ZBTB20* missense variants has increased the total number of patients with *de novo ZBTB20* missense variants to 19 and has allowed further refinement of the Primrose syndrome phenotype (supplementary table 1). Major clinical features, reported in >90% of patients with Primrose syndrome, include an intellectual disability (most frequently in the moderate range), with abnormal findings on brain MRI scan (most frequently abnormalities of the corpus callosum). Patients have a characteristic facial appearance consisting of a prominent forehead, deep set eyes, down slanting and often narrow palpebral fissures, small mouth, thin upper lip, pointed chin and large fleshy ears which may be posteriorly rotated.

Other likely clinical associations of Primrose syndrome, reported in 50-90% of patients with Primrose syndrome, include hearing loss (15/18, 83%), hypotonia (14/18,

78%), cryptorchidism in males (6/8, 75%), macrocephaly (13/18, 72%), behavioral issues (10/18, 56%) and dysplastic or hypoplastic nails (8/14, 57%). In addition, distal muscle wasting, abnormalities of glucose metabolism, contractures and ectopic calcification of the pinnae were reported in at least 80% of adult patients. None of these four clinical features were reported in the five patients in the current study. However, our oldest patient was only 13.4 years and so it is possible that our five patients may still develop these. Of interest, given that abnormalities of glucose metabolism are associated in older individuals with Primrose syndrome and the association between abnormalities of glucose metabolism and increased BMI, the BMI was ≥2SD in two of the three individuals older than ten years and for whom data were available. In contrast, in the seven individuals younger than 10 years for whom data were available, only one had a BMI ≥2SD.

Based on our findings and data from the fourteen previously reported patients with missense variants, our practice is to ensure appropriate learning and behavior support is in place and to refer to physiotherapy for management of hypotonia and/or contractures. We undertake a hearing evaluation for all our patients. In addition, until there are longitudinal data, we are screening our patients for both abnormalities of glucose metabolism and thyroid abnormalities.

All five variants in the current study occurred *de novo.* One variant was mosaic. This has implications for the counselling of families with regard to recurrence risk. We would counsel a 1% recurrence risk where a child has a *de novo* constitutive variant, with no evidence of mosaicism, reflecting the possibility of germline mosaicism and we would offer prenatal testing in future pregnancies. In contrast, the risk of recurrence of a *de*

*novo* mosaic variant is very low (background rate) and invasive prenatal testing for future pregnancies would not be recommended.

Although the current study has allowed further refinement of the Primrose syndrome phenotype, the total number of patients and, in particular, the number of adults with Primrose syndrome remains small. However, as exome/genome sequencing becomes more accessible, it is likely that additional individuals, of all ages, will be identified with Primrose syndrome. This will result in an improving understanding of the Primrose syndrome phenotype, a greater knowledge of the long term syndrome complications and the implementation of optimal, consistent, evidence-based management.

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**WEB RESOURCES**

Centers for Disease Control and Prevention (CDC); https://www.cdc.gov/

Decipher; https://decipher.sanger.ac.uk

ExAC; http://exac.broadinstitute.org

GnomAD; http://gnomad.broadinstitute.org

Online Mendelian Inheritance in Man (OMIM); https://www.omim.org

WHO child growth standards; https://www.who.int/childgrowth/standards/en/

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**Figure 1 a)** schematic of *ZBTB20* showing the BTB domain (blue) and the four C2H2 zinc finger domains (red). Variants identified in the current study are shown above the line and previously reported variants are shown below the line; **b)** facial appearance of five individuals with *ZBTB20* variants and, **c)** sagittal brain MRI images from two of the patients demonstrating abnormalities of the corpus callosum.